(ii) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (c)(3)(ii) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the requirements prescribed in §113.300 and in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in §113.33(a) and the cat safety test prescribed in §113.39(b) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ or plaque forming units per dose.

[44 FR 58899, Oct. 12, 1979, as amended at 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

EFFECTIVE DATE NOTE: At 72 FR 72564, Dec. 21, 2007, §113.315 was amended by removing paragraph (c)(4) and redesignating paragraph (c)(5) as paragraph (c)(4), effective Jan. 22, 2008.

§113.316 Canine Parainfluenza Vaccine.

Canine Parainfluenza Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five canine parainfluenza susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Nasal swabs shall be collected from each dog on the day the first dose of vaccine is administered and individually tested on susceptible cell cultures for the presence of canine parainfluenza virus. Blood samples shall also be drawn and individual serum samples tested for neutralizing antibody. Dogs shall be considered susceptible if all swabs are negative for virus isolation and if all serums are negative for canine parainfluenza antibody at a 1:2 final dilution in a constant virus-varying serum neutralization test using 50 to 300 $TCID_{50}$ of canine parainfluenza virus.

(2) A geometric mean titer of vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be administered a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used to confirm the dosage administered. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Three to 4 weeks after the final dose of vaccine, all dogs shall be bled for serum antibodies and nasal swabs shall be collected for canine parainfluenza virus isolation. On the same day, all vaccinates and controls shall be challenged with canine parainfluenza virus furnished or approved by Animal and Plant Health Inspection Service.

(4) The rectal temperature of each dog shall be taken and the presence of respiratory or other clinical signs of canine parainfluenza virus infection noted and recorded each day for 14 consecutive days postchallenge. Nasal swabs shall be collected from each dog

each day for at least 10 consecutive days postchallenge. Individual swabs shall be tested for virus isolation by culture in canine parainfluenza virus susceptible cells for at least 7 days. Results shall be evaluated according to the following criteria:

(i) If five of five controls have not remained seronegative at a final serum dilution of 1:2 during the prechallenge period, the test is inconclusive and may be repeated.

(ii) If more than one vaccinate shows febrile response, respiratory or other clinical signs of canine parainfluenza virus infection; or, if less than 19 of 20 vaccinates show serum neutralization titers of 1:4 or greater; or, if there is not a significant reduction in virus isolation rate in vaccinates when compared with controls, the Master Seed is unsatisfactory.

(5) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest: *Provided*, That five of five vaccinates and five of five controls shall meet the criteria prescribed in paragraph (b)(4) of this section.

(6) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer at least $10^{0.7}$ greater than that used in the immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ per dose.

(2) [Reserved]

[50 FR 436, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

EFFECTIVE DATE NOTE: At 72 FR 72564, Dec. 21, 2007, §113.316 was amended by removing paragraph (b)(5) and redesignating paragraph (b)(6) as paragraph (b)(5), effective Jan. 22, 2008.

§113.317 Parvovirus Vaccine (Canine).

Parvovirus Vaccine recommended for use in dogs shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in §113.300 and the requirements in this section.

(b) The Master Seed shall be tested for reversion to virulence in dogs using a method acceptable to Animal and Plant Health Inspection Service. If a significant increase in virulence is seen within five backpassages, the Master Seed is unsatisfactory.

(c) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Twenty-five canine parvovirus susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine parvovirus to determine susceptibility. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus-varying serum neutralization test in cell culture using 50 to 300 $TCID_{50}$ of canine parvovirus.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 dogs used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the dosage calculations, five replicate virus titrations